Control of DNA dehybridization via nanoparticle antennas for antisense gene therapy

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a. Scientific and Technical Objectives

The goal of this proposal is to develop the use of a nanoscale interface to protein translation machinery to control translation. External magnetic fields excite the nanoscale interface and thus switch on translation of a protein. This technique would enable time-specific control of protein expression, and would enable new techniques in disease diagnosis and therapy. These objectives are in line with the original proposal.

b. Approach

The approach proposed is to develop a method for controlling translation using nanoparticles as the nanoscale interface:

1) Nanoparticles decorated with antisense DNA can turn off translation. The antisense DNA has a sequence such that it can hybridize to the mRNA over the start codon and ribosome binding sequence. "Blocking" of these sections is known to inhibit translation by preventing the translation machinery (ribsome and associated factors) from binding to the mRNA.

2) External fields heat the nanoparticle. This is similar to techniques already developed, hyperthermia and photothermal therapy, where external fields heat the nanoparticles.

3) Heat from the nanoparticle is transferred to the DNA, causing it to dehybridize from the mRNA. This exposes the start codon and ribosome binding sequence. Because the alternating magnetic field heats only the magnetic particles, only this DNA is denatured.

4) Translation machinery can now bind to the mRNA again, so protein is translated again.

We are implementing this technique on a commercially available in vitro translation system. The protein expressed is green fluorescent protein, GFP, which provides a fluorescent measure of expression. These approaches are still in line with the original proposal.

c. Concise Accomplishments

1) Determine optimal antisense DNA-Au ratio for shutting off translation.

Nanoparticle-DNA conjugates were used to shut off translation. We determined surface chemistries and concentration ranges so that the shut-down was specific.

2) Determine optimal field conditions for heating nanoparticles and subsequent dehybridization of DNA.

We determined that heating of nanoparticles by laser pulses was the best approach for heating the nanoparticles locally. However, heating can reach very high temperatures at the nanoparticle surface and could thus sustain damage to the biomolecules. We are still determining optimal conditions by lower laser powers.

3) Control of translation via nanoparticle-antisense strand

Once the optimal power ranges are determined, we will use heating of the nanoparticles to control translation.

d. Expanded Accomplishments

1) Determine optimal antisense DNA-Au ratio for shutting off translation.

First, we determined surface treatments of the nanoparticles that minimize non-specific interference with the translation process. This would verify that the nanoparticles themselves do not shut down

translation artificially Figure 1 shows expression of GFP (as measured by fluorescence) as a function of nanoparticle concentration, where the nanoparticles do not have any antisense DNA attached to them at all. Nanoparticles with charged ligands (BPS, black squares) tend to non-specifically inhibit translation, but nanoparticles treated with neutral molecules such as polyethylene glycol do not (red circles). In addition, we determined concentration ranges which are suitable for using these particles.

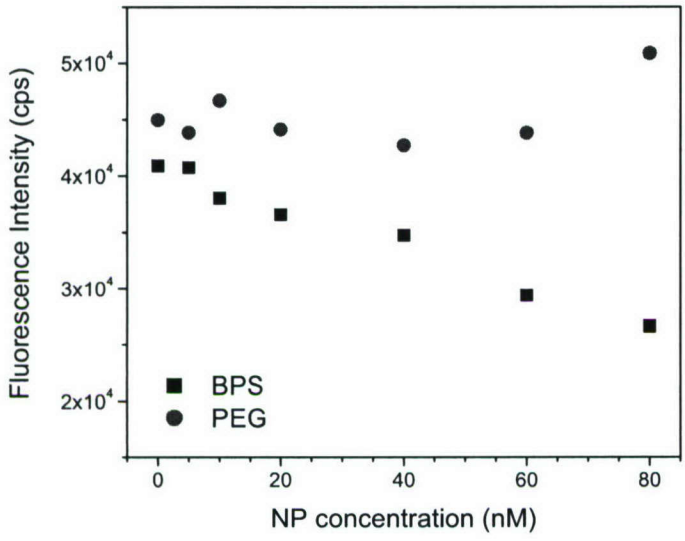

Figure 1. 13nm Au nanoparticles (NP) with no antisense DNA can inhibit translation depending on surface treatment. NPs with the ligand BPS does affect translation of GFP (black) while those with PEG do not (red).

Next, we determined optimal mRNA/DNA ratios for shutting off translation. We did this relative to DNA conjugated to a non complementary, or "nonsense" sequence to verify that the effect was specific. Figure 2 shows GFP expression when exposed to 13nm Au NPs conjugated to antisense DNA (red). As the concentration is increased, the expression of GFP decreases. For Au NPs conjugated to the nonsense strand, the GFP expression is not affected, demonstrating that the effect is specific. This is reported as a DNA:mRNA ratio (x-axis). Therefore, there is a range of DNA/mRNA ratios over which the antisense effect is observable and also specific.

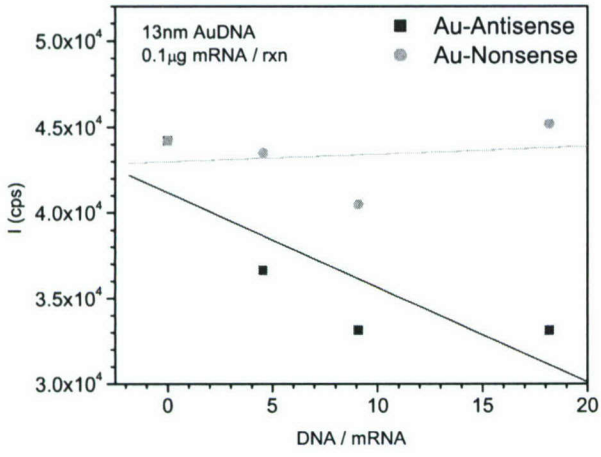

Figure 2. GFP expression when exposed to NPs conjugated to antisense DNA (red) and nonsense DNA (green).

2) Determine optimal field conditions for heating nanoparticles and subsequent dehybridization of DNA.
and
3) Control of translation via nanoparticle-antisense strand

We determined that the optimal method for heating nanoparticles was by ultrafast lasers. We were able to synthesize gold particles that could be heated to extremely high temperatures by a laser pulse. For example, we synthesized gold nanorods that could be excited strongly in the infrared (800nm), and could induce high enough temperatures to melt the nanorods in solution. Because the rest of the solution was relatively unaffected, the heating was determined to be local. However, these temperatures were unable to not sustain damage to DNA so we are still determining appropriate (lower) power ranges. We are still determining optimal conditions which would thus allow us to achieve objective (3) of controlling translation reversibly.

- **Problems/Issues**

Surface treatments that prevent non-specific antisense activity of the NPs themselves can also inhibit DNA functionality. Because of this, DNA conjugation chemistry may have to be improved. In addition, issues with magnetic field heating in our setups were not sufficient for DNA dehybridization, so laser

heating was pursued, and shown to be a viable route. However, as mentioned above, optimal conditions for heating still need to be determined.

g. Required Viewgraph (following page)

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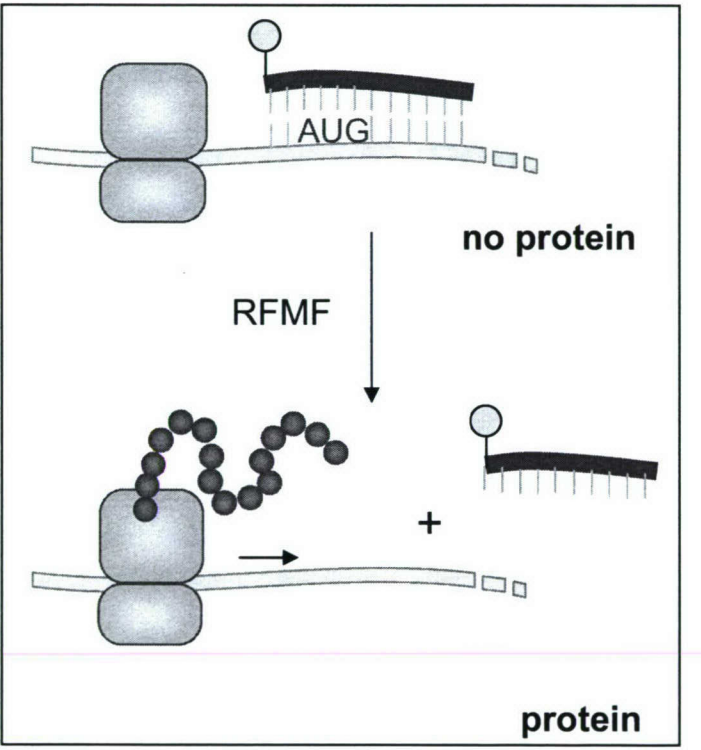

Objective:

•Use external magnetic fields to control translation and thus protein expression

Approach:

•Use antisense DNA linked to nanoparticles to shut off translation
•External magnetic fields heat nanoparticle
•DNA dehybridizes when nanoparticle heated
•Protein translation turned on again

Accomplishments:

•DNA conformation on nanoparticle surface studied
•Antisense:mRNA ratio determined, reversibility with bulk heating demonstrated
•Experimental setup built and optimized

Transitions:

• contact with DARPA/DSO